Do crab spiders perceive Batesian mimicry in hoverflies?

Rohanna Morris and Tom Reader*

School of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, U.K.

*Corresponding author. Email: tom.reader@nottingham.ac.uk. Tel. ++44 (0)115 9513213.

Running title: Do crab spiders perceive hoverfly mimicry?

Key words: Imperfect Batesian mimicry, predator, selection, evolution, aposematism, visual signals, colour patterns.

9 Funding

10 This work was supported by a Natural Environment Research Council PhD studentship award

11 (NE/F008139/1) to RD.

Abstract

Many putative Batesian mimics only approximately resemble their supposed models, and such "imperfect" mimics are readily distinguished from defended species by humans and other vertebrates. One explanation for the existence of imperfect mimics is that the most important predators of many mimics have very different sensory and cognitive abilities from those of a typical vertebrate. In such circumstances, selection for more accurate mimicry, as perceived by humans, may be reduced. Little is known, however, about how invertebrate predators perceive and respond to mimicry in insect prey. Here, we investigate the foraging behaviour of the crab spider *Synema globosum*, an important predator of flower-visiting insects at our field site which frequently encounters both Batesian mimics (hoverflies - Diptera: Syrphidae) and their models (bees and wasps - Hymenoptera). In the field, we found that spiders can distinguish among dipteran and hymenopteran prey taxa, frequently attacking some models and mimics, but avoiding others. Laboratory experiments suggest that some apparently accurate mimic taxa are more likely to be avoided when spiders have prior experience of an aversive wasp model. Avoidance by spiders of black and yellow striped artificial prey suggests visual cues play a role in prey selection, but there was no evidence that olfactory cues are used to identify dangerous or noxious species. Overall, our results provide some support for the hypothesis that invertebrate predator behaviour can generate selection on visual signals in putative Batesian mimics.

Introduction

Batesian mimics are palatable and harmless species which resemble aversive or defended prey, many of which advertise their unprofitability to predators with aposematic warning signals (Bates, 1862; Ruxton et al., 2004). They gain protection from attack by fooling predators into misidentifying them as unprofitable or dangerous. While for over a century Batesian mimicry has been considered a classic example of adaptive evolution, it has long been recognised that many supposed mimics only approximately resemble their putative models (Gilbert, 2005; Sherratt, 2002). This poses the question: if natural selection by predators favours the ever closer resemblance of a mimic to its model, why are so many mimics clearly "imperfect" (Edmunds, 2000)? There are several possible answers to this question (reviewed in Gilbert, 2005), with recent evidence suggesting that selection for perfect mimicry may be relaxed in palatable but relatively unprofitable prey (Penney et al., 2012), and that mimetic accuracy may be traded-off against thermoregulatory costs (Taylor et al., In Review). However, there is no consensus about the most important factors influencing the precision with which mimics resemble their models (Gilbert, 2005).

A widely recognised problem with studies of mimicry is that, whilst many mimics appear imperfect to human or other vertebrate eyes, the predators that are actually responsible for selection of mimetic phenotypes may include taxa (e.g. insects and spiders) with very different sensory and cognitive abilities (Stevens, 2007). Thus, it is entirely possible that real-world predators perceive apparently imperfect mimics as being indistinguishable from aversive or defended prey (Cuthill and Bennett, 1993). As yet, however, there is very little empirical evidence for differences in perception of prey signals among predators, especially in natural situations, and invertebrate predators in particular have been neglected in the literature on mimicry (but see Kauppinen and Mappes, 2003; Rashed et al., 2005). This gap in our knowledge, combined with the fact that we know almost nothing about the relative importance of different current and historical causes of selection on

55 warning and mimetic patterns in natural populations of invertebrates, seriously limits our ability to
56 test hypotheses about the evolution of aposematism and mimicry.

57 It was noted as far back as the origin of the theory of Batesian mimicry that a wide range of
58 predators avoided aposematic butterflies including dragonflies, mantids and flies (see Carpenter and
59 Ford, 1933), but to date the majority of studies of predator perceptions of aposematism and
60 Batesian mimicry have focussed on birds (e.g. Dittrich et al., 1993; Ham et al., 2006; Mostler, 1935)
61 and other vertebrates (e.g. Hetz and Slobodchikoff, 1988; Nonacs, 1985; Osorio et al., 1999). Birds
62 have been seen as important predators of a wide range of aposematic and mimetic prey taxa, as well
63 as being easily trained and having a predictable behavioural repertoire, making them ideal study
64 species. Early experiments demonstrated that avian predators have the potential to select for
65 Batesian mimicry, but that they are not fooled by relatively imperfect mimics (Mostler, 1935). These
66 experiments showed that the more closely hoverflies (Diptera: Syrphidae) appeared to mimic
67 hymenopteran models, the more protection they received from predation. They also showed that
68 birds were less likely to attack certain mimics following experience with their proposed
69 hymenopteran model. Overall, these experiments suggested that birds were generally deceived by
70 mimics, but that the extent of protection enjoyed was dependent on the closeness of the mimic's
71 resemblance to its model.

72 Although birds demonstrate some differences from humans in the perception of prey signals (Bain et
73 al., 2007; Dittrich et al., 1993), and may have some cognitive and sensory limitation which impact on
74 their ability to identify prey correctly (Chittka and Osorio, 2007; Kikuchi and Pfennig, 2010), many
75 mimetic hoverfly species appear to gain little protection from birds as a result of their resemblance
76 to their hymenopteran models (Dlusskii, 1984). This would suggest that the potential for avian
77 predation to select for inaccurate Batesian mimicry in invertebrate prey is limited. However, mimetic
78 hoverflies are also attacked by many invertebrate predators (Howarth and Edmunds, 2000), and

these species are likely to view the mimetic patterns of their prey in a very different way from humans and other vertebrates.

In spite of the strong evidence for the visual and cognitive abilities of invertebrates (e.g. Dyer and Chittka, 2004; Pohl et al., 2008; Tibbetts, 2002), few studies have considered invertebrate predator responses to warning signals and to putative Batesian mimicry. Dejean (1988) found that hunting workers of the ant species, *Odontomachus troglodytes*, not only learned to avoid the warningly coloured larvae of an African chrysomelid beetle, but also retained this behaviour for up to 28 days following an initial costly experience. Similarly, mantids can learn to avoid aposematic prey (Gelperin, 1968), and extend their avoidance to similarly-coloured palatable mimics (Berenbaum and Miliczky, 1984), and palatable, non-mimetic flies given artificial warning signals gained protection from predation by dragonflies (Kauppinen and Mappes, 2003). Taken together, these studies strongly suggest that invertebrate predators have the potential to play a role in selecting for aposematism and Batesian mimicry (but see Rashed et al., 2005 for evidence to the contrary).

Spiders are probably important predators of many aposematic and mimetic invertebrates: we know, for example, that crab spiders (Thomisidae) are a major source of mortality in honeybees (*Apis mellifera*) in some circumstances (Reader et al., 2006). However, we have no direct estimate of the relative contributions of spiders and other predators (e.g. birds) to selection on invertebrate colour patterns, and the extent to which spiders detect and respond to aposematism and mimicry remains almost completely unexplored. Spiders appear to be capable of learning to avoid signals associated with a cost (Huang et al., 2011; Vasconcellosneto and Lewinsohn, 1984), and some species have been shown to have an innate aversion towards defended prey (Nelson and Jackson, 2006). Most spiders are thought to have relatively poor vision, and rely mainly on other senses such as olfaction and mechanoreception when foraging (Herbstein, 2011). Thus, it has been suggested that spiders may select for prey warning signals and mimicry in other modalities (Vasconcellosneto and Lewinsohn, 1984). However, some spiders do rely heavily on vision while hunting. Jumping spiders

(Salticidae) have remarkably acute vision, which may be equivalent to that of some birds (Williams and McIntyre, 1980). They are known to rely on visual cues for the identification of palatable prey, predatory threats and other aspects of their environment (Harland and Jackson, 2004). Furthermore, they have been proposed as important agents of selection on some mimetic signals; juveniles of myrmecomorphic salticids appear to benefit from Batesian mimicry of ants by avoiding attacks from larger salticids (Huang et al., 2011).

Like salticids, crab spiders are cursorial (non-web-building) predators of various aposematic and mimetic invertebrates (Morse, 2007; Reader et al., 2006). Relatively little is known about crab spider vision, but they are considered to be predominantly visual foragers, being able to resolve objects at a resolution similar to that achieved by the compound eyes of some insects (Schmalhofer & Casey, 1999). Observations of the crab spider *Misumena vatia* suggest that it relies heavily on mechanoreceptors for prey detection, and may attack prey relatively indiscriminately (Morse, 2007). The ability of crab spiders, however, or any other spiders, to detect or respond to differences between defended and undefended, or mimetic and non-mimetic prey, has not been examined in detail (but see Tyshchenko, 1961). Hence, the potential of spiders to select for Batesian mimicry remains unexplored, and the consequences of the differences between their sensory and cognitive abilities and those of vertebrates for the evolution of mimetic accuracy in prey such as hoverflies are unknown.

In this study, with a series of field and laboratory experiments, we explore the potential of a crab spider (*Synema globosum* Franganillo) to discriminate among aposematic, defended Hymenoptera and visually mimetic hoverflies. We ask whether venomous wasps and bees are aversive to spiders, and whether prior experience with such species makes spiders more likely to avoid their supposed Batesian mimics. Finally, we ask whether any discrimination among prey types is achieved through the use of visual or olfactory cues. Our ultimate objective is to shed light on the hypothesis that

predators with relatively poor visual acuity compared with vertebrates could be responsible for the evolution of approximate or imperfect visual mimicry in their prey.

Methods

Study System

We examined behavioural responses of *S. globosum* to common aposematic and mimetic prey, and artificial models of prey, at our field site in Sobreda de Caparica, Lisboa, Portugal (38°33'67"N, 009°11'34"W). Adult *S. globosum* are the most abundant flower-dwelling predators at our site in spring (Ibarra, 2013), and are frequently observed attacking flower-visiting arthropods, including a range of mimetic hoverflies, and other non-mimetic flies. Although *S. globosum* frequently kills honeybee workers (Reader et al., 2006), we have not observed it attacking any of the venomous social wasps (Hymenoptera: Vespidae) which visit flowers to feed on nectar and/or hunt for prey, the most common of which is the paper wasp *Polistes dominula* (also referred to as *P. dominulus*). *P. dominula* appears to be a threat to *S. globosum*: spiders were attacked and consumed by wasps when kept in captivity together.

For our experiments, we collected individuals of seven species of Diptera and two species of Hymenoptera from flowers on which *S. globosum* is known to forage (Figure 1; Table 1). Four of the dipteran species have black and yellow or orange stripes on the abdomen, and appear to be mimics of vespid wasps such as *P. dominula*, one is apparently a honeybee mimic, and two are apparently non-mimetic flies. Excluding the honeybee mimic (*Eristalis tenax*), we assigned the dipteran species a rank according to the accuracy with which human volunteers perceived they mimicked *P. dominula*. Twenty seven students at the University of Nottingham were shown one photograph selected at random from three high-resolution colour photographs of *P. dominula*, and one of three photographs (again, selected at random) of each of the six fly species. We used the best images of each species to which we had access, but the scale, lighting conditions etc. of these images varied as was not always known. Volunteers were asked to rank the fly species in the photographs in order of resemblance to *P. dominula*. We did not emphasise the importance of any particular cues/criteria in scoring resemblance.

Experiment 1: Prey Preference

A field experiment was carried out to determine if crab spiders distinguish among prey types, and to establish the palatability of the defended model species (*P. dominula* and *A. mellifera*). Between 0900 and 1800 on 19 sunny days between April 3rd and May 5th 2008, live model and mimic species were offered to *S. globosum* found foraging on flowers in the field and their subsequent response recorded. Individuals of seven prey species (Table 1) were caught with a sweep net no more than 24 hours before the experiment and temporarily stored in specimen tubes. Immediately before the experiment, each individual was stunned using carbon dioxide gas, its wings were removed, and a piece of very fine metal wire (350 mm long and 0.125 mm in diameter) tied between its head and thorax. The removal of the wings ensured that the prey were easier to manipulate, and minimised the extent of any auditory or behavioural cues which might influence spider behaviour. Each individual was suspended on its wire from a 30 cm wooden stick and offered to a different spider (n = 180; 139 females and 41 males). An individual of a randomly selected prey taxon was "dangled" 5 - 10 mm above the centre of a haphazardly selected flower with a resident spider. Random selection of prey types meant that sample sizes were not necessarily equal. Individuals were dangled for 5 min or until they were killed by the spider. In addition to whether prey were killed and consumed by spiders, seven distinct spider behaviours were recorded during trials (Table 2). During the course of Experiment 1, it became clear that "retreat" actually encompassed two distinct behaviours, one of which was subsequently redefined "bungee".

Experiment 2: Can Spiders Learn Aversion to Mimetic Prey?

In order to control the previous experiences and hunger of the crab spiders, all subsequent experiments were carried out under laboratory conditions at our field station. Spiders used in laboratory experiments were collected from flowers in the field, stored individually in 9 cm Petri dishes and kept on a natural light:dark cycle. Prior to their use in experiments, they were starved for at least four days to increase their motivation to hunt.

The first laboratory experiment tested whether prior experience with an aposematic, aversive model species affected spider behaviour when subsequently presented with mimetic and non-mimetic palatable prey. Field observations and the results of Experiment 1 indicated that *P. dominula* and hoverfly mimics of wasps were the most suitable species for this test: whilst *S. globosum* readily attacks and consumes *A. mellifera*, it rarely if ever attacks *P. dominula*, and in fact often flees from a flower upon encountering this species. Thus, we tested 1) whether spiders exposed to *P. dominula* were less likely to attack wasp mimics subsequently, and 2) whether any such acquired aversion to wasp mimics was stronger towards hoverfly species which are considered by humans to be more accurate mimics.

Over 27 days in April – June 2008 and March – May 2009, spiders (n = 259; 188 females and 71 males) were randomly allocated to either "wasp" or "no-wasp" treatments and placed in a clean Petri dish under a desk lamp with a 60W bulb for 10 min. Individuals in the wasp treatment were housed with a live adult *P. dominula* (with wings removed). In a few cases, spiders were injured or killed by wasps, in which case they were discarded. After 10 min, spiders were removed from dishes and placed onto a fresh flower of *Chrysanthemum segetum* standing in a sample tube filled with water, in a Perspex experimental arena (30 cm x 20 cm x 20 cm). *C. segetum* was chosen because crab spiders were often seen foraging on it the field and it did not wilt easily. The spider was given 10 min to settle on the flower, after which a fly specimen from one (randomly selected for each trial) of six species (four wasp mimics and two non-mimics; see Table 1) was dangled above the centre of the flower as above. Random selection of treatments for individual spiders meant that sample sizes were not necessarily equal. Spider behaviours were recorded as before (Table 2). On rare occasions, spiders did not settle on the flower; these individuals were discarded. For this experiment, prey were frozen until dead immediately prior to being presented to spiders, in order to remove mechanosensory stimuli. Preliminary tests revealed that spiders were equally likely to attack live and dead prey.

Experiment 3: Visual Cues

The next experiment was designed to establish whether *S. globosum* relies on visual cues whilst hunting and whether, after experience with a wasp, they show aversion towards visual signals designed to mimic the black and yellow banding on vespid abdomens. Over 15 days in May 2008 and in March and April 2009, we presented spiders with dead flies and artificial "prey" consisting of 10 mm long cylindrical pieces of modelling clay (Staedtler® Fimo®). Both real and artificial prey were manipulated to produce mimetic and non-mimetic forms. Artificial prey were either black clay with black stripes, yellow clay with black stripes or yellow clay with yellow stripes. All stripes were painted using a fine brush and Revell® enamel model paint (yellow matt #15 and black matt #8). A groove cut approximately 2 mm from one end of each cylinder acted as the 'neck' to allow metal wire to be fastened in place (as above). The flies used were the hoverfly *C. intermedium*, which was deemed the most accurate mimic of *P. dominula* by human volunteers, and the non-mimetic fly *F. canicularis* (Table 1). Their wings and legs were removed. Two "altered" forms of *C. intermedium* were also used for comparison with artificial prey, and to control for possible effects of paint on spider behaviour: they had either their yellow markings or their black markings painted over using black paint (as above). Spiders (n = 98; 69 females and 29 males) were exposed to wasps as in the wasp treatment in Experiment 2, and then transferred to flowers in the experimental arena. They were offered artificial prey of a randomly selected type, as above, for 3 min or until the spider attacked. Spider behaviour was recorded as before (Table 2). Random selection of the prey type for each individual spider meant that sample sizes were not necessarily equal.

Experiment 4: Olfactory Cues

The final experiment was designed to determine whether *S. globosum* uses olfactory cues whilst hunting, and whether there is olfactory mimicry in the apparently accurate visual wasp mimic *C. intermedium*. All prey in this experiment were cylindrical pieces of black modelling clay. Each cylinder was randomly assigned an odour treatment: wasp, honeybee, wasp mimic or nothing.

Odour was transferred using a similar method to that used by Wood and Ratnieks (2004). Each cylinder was placed in a sample tube with a live wasp (*P. dominula*), honeybee (*A. mellifera*), wasp mimic (*C. intermedium*) or nothing, and shaken for ten seconds. We shook the tube sufficiently to prevent the live animals from avoiding contact with the artificial prey, but not so vigorously that they were obviously harmed. The experiment was carried out over 15 days in April 2010. Each spider (n = 238; 161 females and 77 males) was randomly assigned to a "wasp" or "no-wasp" treatment, and exposed to *P. dominula* for 10 minutes as in Experiment 2. Spiders were then transferred to a flower in the experimental arena and offered a prey cylinder from a randomly chosen odour treatment for three minutes, or until it was attacked. Behaviours exhibited by the spiders during trials were recorded as before (Table 2).

Statistical Analysis

For analysis, we characterised spider responses to prey in two different ways. First, where possible, we considered whether or not prey were killed in a trial as a binary response variable. In experiments with dead or artificial prey, we considered whether or not prey were attacked at least once, instead of whether they were killed. Second, we considered the frequency with which each different behaviour (Table 2) occurred in each trial. Because the frequencies of some behaviours were clearly correlated, we used Principal Components Analysis (PCA) to derive a smaller number of uncorrelated response variables with which to describe these data. We extracted all Principal Components (PCs) with Eigenvectors of greater than 1.0 for use in subsequent analysis.

The effects of spider sex, prey taxon or type, treatment (exposure to *P. dominula*) and prey mimetic accuracy on the two types of response variable were analysed using either binomial or Gaussian generalised linear models (GLMs) or linear mixed effects models (GLMMs). For all experiments, we fitted GLMs, in which spider sex and prey taxon/type were fixed factors, and there were no random effects. Where appropriate, treatment was also fitted as a fixed factor. In Experiments 1 and 2, we explicitly tested the effect of mimetic accuracy using a GLMM, with prey taxon as a random factor

and prey mimetic accuracy (ranked) as a fixed covariate. Model fitting and simplification followed Zuur et al. (2009). The significance of fixed effects was assessed by testing the effect of deleting terms sequentially, starting with the highest order interactions, from a saturated model with Likelihood Ratio, F-ratio or Chi-squared tests. PCA and model fitting was done using the packages glmer and stats in R Version 3.0.1 (R Core Team, 2015). Post hoc comparisons of prey taxa were performed using the package phia. Where relevant, planned comparisons were applied with a custom contrast matrix.

Results

Experiment 1: Prey Preference

When offered prey in the field, spiders were significantly more likely to kill and consume some taxa than others (binomial GLM: $\chi^2_7 = 89.434$, $p < 0.001$; Figure 2). Spiders never killed *P. dominula*, but readily consumed most dipteran prey, except for the honeybee mimic *E. tenax*. Female spiders were significantly more likely to kill prey (62.6 % of 139) than males (25 % of 40) ($\chi^2_1 = 8.6142$, $p = 0.003$), but there was no interaction between sex and prey taxon ($\chi^2_7 = 7.953$, $p = 0.337$). Considering only the data for the wasp mimics and control fly species, the effect of spider sex was no longer significant (GLMM: $\chi^2_1 = 1.579$, $p = 0.209$), spiders were not significantly more likely to kill less accurate mimics ($\chi^2_1 = 0.295$, $p = 0.587$), and there was no interaction between sex and accuracy ($\chi^2_1 = 1.480$, $p = 0.224$).

Principal Components Analysis (PCA) of spider behaviour yielded two Principal Components (PCs) with Eigenvalues greater than one (Table 3). The first (PC1) correlated positively with the frequencies of all behaviours except “attack” and “left flower”, and hence can be interpreted as a general index of activity exhibited by spiders when they encountered prey. The second (PC2) correlated positively with the number of attacks observed, and negatively with the number of times the spider left the flower upon which it was sitting, and hence contrasts the most positive and the most negative reactions by spiders to prey, with positive scores indicating positive reactions.

Spiders behaved differently towards different prey taxa. Scores on PC1 and PC2 were significantly different among prey taxa (PC1: $F_{(7, 171)} = 6.204$, $p < 0.001$; PC2: $F_{(7,170)} = 2.591$, $p = 0.015$), with spiders being most active and least likely to react positively to *P. dominula*, and least active but most likely to respond positively to the non-mimic *Fannia canicularis* and the relatively inaccurate wasp mimic *Sphaerophoria scripta* (Figure 3). There was a significant difference between male and female spiders in PC2 scores ($F_{(1,170)} = 10.396$, $p = 0.002$), with males being less positive towards prey (mean

= -0.460; SEM = 0.196) than females (mean = 0.132; SEM = 0.075), but not in PC1 scores ($F_{(1,170)}$ = 0.013, p = 0.910). There was no interaction between the effects of sex and taxon on either PC (PC1: $F_{(7,163)}$ = 1.717, p = 0.108; PC2: $F_{(7,163)}$ = 1.879, p = 0.076). Considering only the data for the wasp mimics and control fly species, male spiders were both more active (GLMM for PC1: χ^2_1 = 8.433, p = 0.004) and less positive towards prey (PC2: χ^2_1 = 7.302, p = 0.007). However, there was no significant effect of mimetic accuracy on either measure of behaviour (PC1: χ^2_1 = 0.136, p = 0.713; PC2: χ^2_1 = 0.001, p = 0.999) and no interaction between accuracy and sex (PC1: χ^2_1 = 0.304, p = 0.581; PC2: χ^2_1 = 3.124, p = 0.077).

Experiment 2: Can Spiders Learn Aversion to Mimetic Prey?

When offered hoverflies with varying levels of mimetic accuracy, and non-mimetic flies, in the laboratory, spiders with prior experience of *P. dominula* were slightly less likely to kill prey (63.6 % of 129) than spiders without such experience (72.3 % of 130), but this effect was not significant (binomial GLM: χ^2_1 = 2.354, p = 0.125). The probability that a spider killed a prey item was significantly affected by taxon (χ^2_1 = 36.714, p < 0.001; see Figure 4), with the relatively accurate mimic *C. intermedium* being killed least often, and the non-mimetic fly *F. canicularis* being killed most often. Spider sex did not significantly affect the probability of prey being killed (χ^2_1 = 3.145, p = 0.076). None of the interactions among the main effects on the probability that spiders killed a prey item was significant (p > 0.3 in all cases). It is worth noting that, whilst most prey taxa were attacked at similar frequencies in the two treatments, the most accurate mimic taxon was about half as likely to be attacked by spiders if they had previously been exposed to *P. dominula*. However, when the data were reanalysed with a mixed model, there was no significant effect of mimetic accuracy on the probability of prey being killed (binomial GLMM: χ^2_1 = 2.674, p = 0.102) and no significant interactions involving accuracy (p > 0.1 in all cases). Tests of the other main effects and interactions in the mixed model yielded qualitatively identical results to the GLM (results not shown).

PCA of spider behaviours in Experiment 2 produced three PCs with Eigenvalues greater than one (see Table 3). The first two PCs were similar to those produced in Experiment 1: the first correlated positively with all behaviours except "attack", and the second contrasted the frequency of attacks with the frequency with which spiders left flowers. The third PC correlated most strongly (positively) with "bungee", a behaviour which was not distinguished from "retreat" in Experiment 1.

Analysis which modelled the effect of prey taxon as a fixed factor (GLMs) showed that there were no significant effects of prior exposure to *P. dominula*, spider sex or prey taxon, or their interactions, on PC1 (Table 4; Figure 5). There were, however, significant main effects of prey taxon and treatment on PC2 (Figure 6). Spiders behaved most positively towards *S. ribesii* and most negatively towards *C. intermedium*. In addition, spiders were less likely to attack prey, and more likely to flee them, if they had previously been exposed to wasps. There were no significant interactions involving these main effects on PC2. The behaviours captured by PC3 showed a complex response to the different explanatory variables, with a significant three-way interaction in addition to a main effect of taxon (Fig S1, supplementary information). In general, spiders were least likely to "bungee" (i.e. had the lowest PC3 scores) in response to *C. intermedium*, and most likely to exhibit this behaviour in response to *S. ribesii.* The significant three-way interaction reflects contrasting patterns in male and female PC3 scores, particularly towards *S. ribesii*, *S. scripta* and *E. balteatus*: prior exposure to wasps had a relatively positive effect on the male propensity to "bungee" in response to S. *ribesii* and *E. balteatus*, and a relatively negative effect on the male propensity to "bungee" in response to *S. scripta*, when compared to the equivalent effects of exposure on females.

The mixed model analysis, which included a fixed effect of mimetic accuracy, did not reveal any significant main effects on any of the principal components, but did indicate a significant interaction between treatment and mimetic accuracy: spiders which had been previously been exposed to the aversive model species were relatively more active in response to more accurate mimics, in comparison with less accurate or non-mimetic prey (Figure 5).

Experiment 3: Visual Cues

When spiders previously exposed to *P. dominula* were offered real and artificial prey whose visual appearance was more or less similar to the aversive model species, the probability that they would attack was significantly affected by prey type (binomial GLM: $\chi^2_6 = 21.173$, p = 0.002; Figure 6). Unmanipulated hoverfly mimics with black and yellow stripes (*C. intermedium*) and stripy artificial prey were the least likely to be attacked. According to planned comparisons, artificial prey without stripes were significantly more likely to be attacked than those with stripes. Hoverflies with their yellow or black stripes painted black were more likely to be attacked than unpainted equivalents, although not significantly so. Unmanipulated, non-mimetic control flies were the prey type most likely to be attacked. Spider sex (GLM: $\chi^2_1 = 0.034$, p = 0.854), and the interaction between sex and prey type (GLM: $\chi^2_6 = 8.212$, p = 0.223), did not significantly affect the probability of attack.

As in Experiment 2, PCA produced three PCs with Eigenvalues greater than one, the first two of which had similar correlations with the raw variables describing spider behaviour (Table 3). None of these PCs was significantly affected by prey type (Gaussian GLM for PC1: $F_{6,89} = 0.941$, p = 0.470; PC2: $F_{6,89} = 1.967$, p = 0.079; PC3: $F_{6,89} = 0.750$, p = 0.611), although PC2 (contrasting the propensity to attack with the tendency to leave the flower) showed patterns consistent with the above analysis of spider attacks: stripy artificial prey scored lower than those without stripes, and the same was true for hoverflies, whilst unmanipulated control flies had the most positive scores (Fig S2, supplementary infromation). There were no significant effects of spider sex ($F_{1,88} < 3.0$, $p > 0.05$) or the interaction between sex and prey type ($F_{6,82} < 1.5$, $p > 0.1$) on PC scores.

Experiment 4: Olfactory Cues

When offered artificial prey bearing the odour of models (*P. dominula* and *A. mellifera*) and mimics (*C. intermedium*), or no odour, in the laboratory, there were no significant effects of prior experience with *P. dominula* (binomial GLM: $\chi^2_1 = 1.689$, p = 0.194), odour type ($\chi^2_3 = 1.609$, p = 0.657) or spider

sex (χ^2_1 = 1.568, p = 0.211) on the probability that spiders would attack. Neither were there any significant effects of any interactions among these effects on the probability that spiders would attack prey ($\chi^2_{1 \text{ or } 3}$ < 5.0, p > 0.1).

As in Experiments 2 and 3, PCA produced three PCs with Eigenvalues greater than one, the first two of which had similar correlations with the raw variables describing spider behaviour (Table 3). None of these PCs was significantly affected by odour type or prior experience with *P. dominula* (Table 5). However, PC1, but not PC2 and PC3, was significantly affected by spider sex, with male spiders scoring more highly, indicating higher frequencies of most behaviours (especially display, retreat and approach) than were seen in females. There were no significant interactions among the effects of prior experience, odour type and sex on any of the PCs.

Discussion

The results of our experiments demonstrate that the crab spider *S. globosum* has clear prey preferences, and suggest that those preferences are influenced by the visual appearance of prey, but not by olfactory cues. In addition, there was limited evidence that prior experience with an aversive model species altered spider behaviour towards more accurate Batesian mimics of that model. Overall our results provide support the idea that invertebrate predators can play a role in the evolution of the visual appearance of their prey, and shed some light on the hypothesis that differences between invertebrate and vertebrate sensory and cognitive processes result in relaxed selection on mimetic perfection in prey which are subject to predation by invertebrates.

Prey preferences

The existence and nature of prey preferences in crab spiders are poorly reported. The large, flower-dwelling species *Misumena vatia* apparently shows very little discrimination among prey types, and the frequencies of prey taxa in its diet are thought simply to reflect variation in prey availability (Morse, 2007). By contrast, our results show that *S. globosum* readily distinguished among prey

386 species in the field, completely avoiding *P. dominula*, whilst killing and consuming nearly all
387 individuals of some dipteran taxa. *S. globosum* therefore has the potential to exert selection
388 pressure on prey phenotypes via the cues it uses to identify prey types. Other studies have shown
389 that even when spiders are able to discriminate among prey types, this discrimination may not affect
390 selection, because even rejected prey were left irreparably injured or dead (see Vasconcellosneto
391 and Lewinsohn, 1984). In the case of *S. globosum*, however, our observations showed that none of
392 the prey which were bitten were later rejected, and all rejected prey were left unharmed. Therefore,
393 especially given its abundance and likely impact on prey populations (Ibarra and Reader, 2014;
394 Reader et al., 2006), the results of our experiments suggest that *S. globosum* does indeed have the
395 potential to select for visual or other traits in aposematic and mimetic prey.

396 The putative model wasp species *P. dominula* was never attacked in the field experiment.
397 Furthermore, encountering *P. dominula* caused the spiders to become very active, often leaving the
398 flower completely during the prey presentation period. This suggests that *P. dominula* was aversive
399 to *S. globosum*, either because it is unprofitable, distasteful, or dangerous. Birds may avoid vespid
400 wasps because their abdomens are distasteful (Mostler, 1935), but circumstantial evidence points
401 away from this explanation for avoidance of *P. dominula* by *S. globosum*: while other spiders have
402 been shown to attack unpalatable prey before rejecting it moments later (Vasconcellosneto and
403 Lewinsohn, 1984), we have only seen *S. globosum* attack *P. dominula* once (during exposure in the
404 laboratory in Experiment 2), and on this occasion the spider appeared to feed unperturbed until the
405 wasp was completely consumed. Instead, the main deterrent associated with *P. dominula* appears to
406 be its aggressive and/or predatory nature. In the laboratory, it frequently bit or attempted to sting *S.*
407 *globosum*, killing spiders on several occasions, and in the field *P. dominula* is often seen hunting
408 invertebrate prey on or around flowers which harbour *S. globosum*. It is therefore high plausible that
409 learned or evolved avoidance of *P. dominula* is adaptive in *S. globosum*.

In contrast to the results for *P. dominula*, the other aposematic, defended insect we offered to *S. globosum* in Experiment 1 – the honeybee – was readily attacked and consumed, a fact which helps to explain the levels of mortality that spiders impose on honeybees in our field site (Reader et al., 2006). For this reason, we excluded the honeybee and its mimic *E. tenax* from subsequent experiments in which we were interested in responses to mimicry of aversive prey. The honeybee's defences are apparently not a threat to *S. globosum*, which underlines the likely importance of *P. dominula*'s aggressive predatory behaviour, rather than the possession of a sting per se, in determining spider prey choice. The almost complete avoidance of *E. tenax*, which to humans is a good mimic of the honeybee, in Experiment 1 is puzzling. It suggests that, although visual appearance may play a role in some circumstances (as in Experiment 3, for example), other factors affect spider prey choice significantly. Which cues were used to identify *E. tenax* as unpalatable, and the reason why it is unpalatable, remain a mystery which only further experimentation can unlock.

In two of our four experiments, sex had a significant effect on spider responses to prey. There was no evidence that prey preferences were different between males and females, but males were in general less likely to attack prey in the field, and more active (rather "skittish") when presented with artificial prey in the laboratory. These differences could be explained by the pronounced sexual size dimorphism (Blanckenhorn, 2005) that is seen in many spiders, including *S. globosum* (full grown females are about seven times the mass of males; Corcobado et al., 2010). Typically, smaller male spiders have a different diet from larger females (Walker and Rypstra, 2002). Furthermore, males often spend the majority of the time searching for and guarding females and invest less energy and time in foraging for themselves (e.g. Givens, 1978) than females, whose reproductive fitness is closely related to foraging success (Walker and Rypstra, 2002). As a result, male *S. globosum* may have been more interested in fleeing the confines of the experimental arena than they were in finding a meal. Their smaller body size did not, however, obviously constrain prey choice: just like females, males showed a relative preference for the largest popular prey type (honeybees) compared with some of the smaller prey in Experiment 1, for example.

436 *Responses to Batesian mimicry*

437 Our results provide some support for the hypothesis that *S. globosum* perceives and responds to
438 Batesian mimicry in its prey. Results from the laboratory (Experiment 2) showed that, overall, spider
439 attack rates and other measures of behaviour towards hoverflies which mimic wasps did not
440 correlate strongly with mimetic accuracy, as perceived by humans. However, recent prior exposure
441 to the aversive model *P. dominula* resulted in some changes in behaviour which were most marked
442 towards the hoverflies which most closely resemble the model. Whilst the relatively large (c. 50 %)
443 decline in the frequency of attacks by spiders exposed to the model on the best mimic (*C.*
444 *intermedium*) was not significantly different from the change in the attack rate on other prey
445 species, there was a significant interaction between the effects of mimetic accuracy and exposure to
446 the model on spider activity levels. The high levels of spider activity which characterised their
447 response to the model species in Experiment 1 were mirrored in exposed spiders offered relatively
448 accurate mimics (especially *C. intermedium*) in Experiment 2. One explanation for this pattern is that
449 exposed spiders generalised from learned responses to wasp cues, resulting in them avoiding or
450 trying to escape from those particular prey taxa which humans perceive to be accurate Batesian
451 mimics. Hence, although the statistical support for the observed pattern is somewhat equivocal, and
452 we do not know what the effects on prey fitness would be in the wild, our data do provide some
453 tentative suggestions that spiders might respond to Batesian mimicry (see also Tyshchenko, 1961).
454 There are, however, alternative explanations for the observed patterns. Rather than facilitating
455 learning about wasp-related cues, a recent encounter with a potential predator may "prime" *S.*
456 *globosum* for danger, making it more likely to exhibit innate avoidance behaviours in subsequent
457 encounters with certain prey types. This priming might not be specific to *P. dominula*, and might
458 have nothing to do with aposematism or mimicry. We would have to repeat the experiment with a
459 control treatment in which spiders were exposed to a different insect (e.g. one which was not
460 aposematic, and not dangerous) in order to explore the possibility further.

461 Although we found some evidence that mimetic accuracy as perceived by humans may affect spider
462 behaviour, there are several reasons why we might expect spiders to rank mimics differently from
463 vertebrates. In addition to differences in sensory and cognitive capabilities, spiders probably also
464 view their insect prey from a different angle from that typically experienced by birds or humans. In
465 our study, humans evaluated the accuracy of mimics from photographs taken from above, whilst
466 spiders were beneath their prey as they were "dangled" onto flowers. Since prey appear very
467 different from below, this could substantially change the accuracy of any perceived mimicry from
468 the spider's perspective, but the significance of any such change remains unknown without testing
469 the effect of prey orientation on human and spider behaviour.

470 There were other limitations to the ranking exercise we used to assess mimetic accuracy of the
471 insect taxa we used in our experiments. For example, we were not able to standardise the scale of
472 the images we showed to human volunteers, and hence reliable information about body size was
473 not available to them, in contrast to the situation for spiders in the experiments. Overall, therefore,
474 we are cautious about the interpretation of our analyses which included mimetic accuracy as a
475 predictor, and we think the most important message from our study is that spiders responded
476 differently to different prey taxa and different artificial prey types in a way which highlights the
477 *potential* of spiders as agents of selection on colour patterns in mimetic and aposematic insects.

478 *The importance of visual, olfactory and other cues*

479 The results show that *S. globosum* relies at least partly on vision when hunting. While the
480 preferences demonstrated in the field (Experiment 1) may have been influenced by olfactory and/or
481 mechanosensory information associated with live prey, Experiment 3 showed that spiders also
482 discriminated among artificial prey that varied only in colour. The results from Experiment 3 also
483 suggest that *S. globosum* responds to typical aposematic signals: following previous wasp
484 experience, the spiders attacked black and yellow striped artificial prey significantly less often than
485 the completely yellow and completely black artificial prey. It remains unclear whether the important

cue here was variation in hue (yellow versus black) or simply luminance (contrast between dark and light stripes), but evidence for true colour vision in crab spiders (Thomisidae) is limited (Insausti et al., 2012).

There was no evidence that *S. globosum* distinguished among the different odour treatments in Experiment 4. The results thus suggest that crab spiders do not utilise olfactory signals to detect the aversive model *P. dominula* or its mimcs. This could be because the olfactory signals of wasps have not evolved under selection by predators, and are not as readily learned and remembered as aposematic colour signals are thought to be (Stevens, 2007; Svadova et al., 2009). Alternatively, *S. globosum* may not rely heavily on olfactory signals while foraging, as appears to be the case in the closely related crab spider, *Misumena vatia* (Morse, 2007).

The laboratory experiments presented here were designed to eliminate behavioural, auditory and mechanosensory stimuli emitted by prey. Under natural conditions, these stimuli may contribute to decision making by crab spiders, and hence they may play a role in determining the adaptive value of wasp mimicry by hoverflies. There is evidence of behavioural mimicry in some hoverfly species (Rotheray and Gilbert, 2011), but the only published evidence suggests that hoverflies do not mimic the sounds of their hymenopteran models (Rashed et al., 2009). Observations of the behaviour of the *M. vatia* suggest that it depends largely on mechanoreceptors when hunting (Morse, 2007), but we know little about variance in the kind of vibrations produced among the potential prey of crab spiders. In order to understand fully the potential of predators such as crab spiders to influence the evolution of prey morphology and behaviour, further investigation of the importance of cues in each sensory modality is required.

Conclusion

Overall, this study provides evidence that spiders have the ability to differentiate among prey using visual cues, and therefore that they have the potential to exert selection on prey morphology,

510 including perhaps aposematic and mimetic colour patterns. Previous studies of Batesian mimicry in
511 insects have tended to assume that the predators responsible for the evolution of warning patterns
512 and their mimetic equivalents are vertebrates. Given that invertebrates are important predators of
513 flower-visiting insects and several other groups containing aposematic and mimetic species, our
514 results suggest that the evolutionary significance of selection on aposematic and mimetic patterns
515 by invertebrate predators is worthy of more detailed consideration. The very different sensory and
516 cognitive abilities of invertebrate predators such as spiders could easily result in patterns of
517 selection which are not well predicted by human or avian behaviour, and hence our results are
518 broadly consistent with the hypothesis that the perceived degree of perfection of a Batesian mimic
519 may be in “the eye of the beholder” (Cuthill and Bennett, 1993). Crucially, in our community of
520 models and mimics, and in most others, the relative contributions of different types of predator to
521 selection on mimetic patterns remains unknown. The phenotype of a Batesian mimic should
522 represent a net evolved response to selection imposed by all predators. Careful consideration of the
523 importance and selectivity of predation from all sources is therefore needed to understand properly
524 how and why mimetic signals appear as they do.

525 Acknowledgements

526 We thank the administrator of the Quinta de São Pedro Study Centre, Armin Pircher, and his
527 assistant Ben Bödecker, for making the field work possible, and Francis Gilbert, Graeme Ruxton and
528 two anonymous reviewers for their advice.

529

References

Bain RS, Rashed A, Cowper VJ, Gilbert FS, Sherratt TN, 2007. The key mimetic features of hoverflies through avian eyes. Proc R Soc B-Biol Sci 274:1949-1954.

Bates H, 1862. Contributions to an insect fauna of the Amazon valley. Lepidoptera: Heliconidae. Transactions of the Linnean Society of London 23:495-566.

Berenbaum MR, Miliczky E, 1984. Mantids and milkweed bugs - efficacy of aposematic coloration against invertebrate predators. American Midland Naturalist 111:64-68.

Blanckenhorn WU, 2005. Behavioral causes and consequences of sexual size dimorphism. Ethology 111:977-1016.

Carpenter GDH, Ford EB, 1933. Mimicry: Methuen, London.

Chittka L, Osorio D, 2007. Cognitive dimensions of predator responses to imperfect mimicry? Plos Biology 5:2754-2758.

Corcobado G, Rodriguez-Girones MA, De Mas E, Moya-Larano J, 2010. Introducing the refined gravity hypothesis of extreme sexual size dimorphism. Bmc Evolutionary Biology 10.

Cuthill IC, Bennett ATD, 1993. Mimicry and the eye of the beholder. Proc R Soc B-Biol Sci 253:203-204.

Dejean A, 1988. Prey capture by *Camponotus maculatus* (Formicidae - Formicinae). Biology of Behaviour 13:97-115.

Dittrich W, Gilbert F, Green P, McGregor P, Grewcock D, 1993. Imperfect mimicry - a pigeon's perspective. Proc R Soc B-Biol Sci 251:195-+.

Dlusskii GM, 1984. Are dipteran insects protected by their similarity to stinging hymenopterans? Biulleten' Moskovskogo Obshchestva Ispytatelei Prirody Otdel Biologicheskii 89:25-40.

Dyer AG, Chittka L, 2004. Fine colour discrimination requires differential conditioning in bumblebees. Naturwissenschaften 91:224-227.

Edmunds M, 2000. Why are there good and poor mimics? Biological Journal of the Linnean Society 70:459-466.

556 Gelperin A, 1968. Feeding behaviour of praying mantis - a learned modification. Nature 219:399-&.
557 Gilbert F, 2005. The evolution of imperfect mimicry. . In: Fellowes MDE, Holloway GJ, Rolff J, editors.
558 Insect evolutionary ecology Proceedings of the Royal Entomological Society's 22nd
559 Symposium Wallingford, UK.: CABI. p. 231-288.
560 Givens RP, 1978. Dimorphic foraging strategies of a salticid spider (*Phidippus audax*). Ecology 59:309-
561 321.
562 Ham AD, Ihalainen E, Lindstrom L, Mappes J, 2006. Does colour matter? The importance of colour in
563 avoidance learning, memorability and generalisation. Behavioral Ecology and Sociobiology
564 60:482-491.
565 Harland DP, Jackson RR, 2004. Portia perceptions: the Umwelt of an araneophagic jumping spider. .
566 In: Prete FR, editor. Complex Worlds From Simpler Nervous Systems: The MIT Press,
567 Cambridge. p. 5-40.
568 Herbstein ME, 2011. Spider Behaivour: Cambridge University Press, Cambridge.
569 Hetz M, Slobodchikoff CN, 1988. Predation pressure on an imperfect batesian mimicry complex in
570 the presence of alternative prey. Oecologia 76:570-573.
571 Howarth B, Edmunds M, 2000. The phenology of Syrphidae (Diptera): are they Batesian mimics of
572 Hymenoptera. Biological Journal of the Linnean Society 71:437-457.
573 Huang JN, Cheng RC, Li DQ, Tso IM, 2011. Salticid predation as one potential driving force of ant
574 mimicry in jumping spiders. Proc R Soc B-Biol Sci 278:1356-1364.
575 Ibarra HA, 2013. Maintenance of a female-limited colour polymorphism in the crab spider Synema
576 globosum (Aranaea: Thomisidae). University of Nottingham.
577 Ibarra HA, Reader T, 2014. Female-limited colour polymorphism in the crab spider Synema globosum
578 (Araneae: Thomisidae). Biological Journal of the Linnean Society 113:368-383.
579 Insausti TC, Defrize J, Lazzari CR, Casas J, 2012. Visual fields and eye morphology support color vision
580 in a color-changing crab-spider. Arthropod Structure & Development 41:155-163.

581 Kauppinen J, Mappes J, 2003. Why are wasps so intimidating: field experiments on hunting
582 dragonflies (Odonata : Aeshna grandis). Animal Behaviour 66:505-511.
583 Kikuchi DW, Pfennig DW, 2010. Predator Cognition Permits Imperfect Coral Snake Mimicry.
584 American Naturalist 176:830-834.
585 Morse DH, 2007. Predator on a Flower.: Harvard University Press, Cambridge.
586 Mostler G, 1935. Beobachtungen zur Frage der Wespenmimikry. Z Morphol Okol Tiere 29:381-454.
587 Nelson XJ, Jackson RR, 2006. Vision-based innate aversion to ants and ant mimics. Behavioral
588 Ecology 17:676-681.
589 Nonacs P, 1985. Foraging in a dynamic mimicry complex. American Naturalist 126:165-180.
590 Osorio D, Miklosi A, Gonda Z, 1999. Visual ecology and perception of coloration patterns by domestic
591 chicks. Evolutionary Ecology 13:673-689.
592 Penney HD, Hassall C, Skevington JH, Abbott KR, Sherratt TN, 2012. A comparative analysis of the
593 evolution of imperfect mimicry. Nature 483:461-U110.
594 Pohl M, Watolla T, Lunau K, 2008. Anther-mimicking floral guides exploit a conflict between innate
595 preference and learning in bumblebees (Bombus terrestris). Behavioral Ecology and
596 Sociobiology 63:295-302.
597 R: A Language and Environment for Statistical Computing. 2015. R: A Language and Environment for
598 Statistical Computing. R Foundation for Statistical Computing.
599 Rashed A, Beatty CD, Forbes MR, Sherratt TN, 2005. Prey selection by dragonflies in relation to prey
600 size and wasp-like colours and patterns. Animal Behaviour 70:1195-1202.
601 Rashed A, Khan MI, Dawson JW, Yack JE, Sherratt TN, 2009. Do hoverflies (Diptera: Syrphidae) sound
602 like the Hymenoptera they morphologically resemble? Behavioral Ecology 20:396-402.
603 Reader T, Higginson AD, Barnard CJ, Gilbert FS, Behavioural Ecology Field C, 2006. The effects of
604 predation risk from crab spiders on bee foraging behavior. Behavioral Ecology 17:933-939.
605 Rotheray GE, Gilbert FS, 2011. The Natural History of Hoverflies: Forrest Text, Tresaith.

606 Ruxton GD, Sherratt TN, Speed MP, 2004. Avoiding Attack: the Evolutionary Ecology of Crypsis,
607 Warning Signals and Mimicry.: Oxford University Press, Oxford.
608 Sherratt TN, 2002. The evolution of imperfect mimicry. Behavioral Ecology 13:821-826.
609 Stevens M, 2007. Predator perception and the interrelation between different forms of protective
610 coloration. Proc R Soc B-Biol Sci 274:1457-1464.
611 Svadova K, Exnerova A, Stys P, Landova E, Valenta J, Fucikova A, Socha R, 2009. Role of different
612 colours of aposematic insects in learning, memory and generalization of naive bird
613 predators. Animal Behaviour 77:327-336.
614 Taylor CH, Gilbert F, Reader T, In Review. Why many Batesian mimics are inaccurate: evidence from
615 hoverfly colour patterns.
616 Tibbetts EA, 2002. Visual signals of individual identity in the wasp Polistes fuscatus. Proc R Soc B-Biol
617 Sci 269:1423-1428.
618 Tyshchenko VP, 1961. On the relationship between flower spiders of the family Thomisidae and
619 mimetic Diptera and their models. Vestnik Leningradskogo Gosudarstvennogo Universiteta
620 Ser Biologiya:133-139.
621 Vasconcellosneto J, Lewinsohn TM, 1984. Discrimination and release of unpalatable butterflies by
622 *Nephila clavipes*, a neotropical orb-weaving spider. Ecological Entomology 9:337-344.
623 Walker SE, Rypstra AL, 2002. Sexual dimorphism in trophic morphology and feeding behavior of wolf
624 spiders (Araneae : Lycosidae) as a result of differences in reproductive roles. Canadian
625 Journal of Zoology-Revue Canadienne De Zoologie 80:679-688.
626 Williams DS, McIntyre P, 1980. The principal eyes of a jumping spider have a telephoto component.
627 Nature 288:578-580.
628 Wood MJ, Ratnieks FLW, 2004. Olfactory cues and Vespula wasp recognition by honey bee guards.
629 Apidologie 35:461-468.
630 Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM, 2009. Mixed Effects Models and Extensions in
631 Ecology with R.: Springer, .

632

633 Figure 1. Nine insect species offered to crab spiders in experiments. A) The honeybee (*Apis mellifera*)
634 and B) its mimic (*Eristalis tenax*). C) The paper wasp (*Polistes dominula*). Wasp mimics in descending
635 order of accuracy, as perceived by humans: D) *Chrysotoxum intermedium*, E) *Syrphus ribesii*, F)
636 *Sphaerophoria scripta*, and G) *Episyrphus balteatus*. Non-mimetic flies: H) *Sarcophaga carnaria* and I)
637 *Fannia canicularis.* Images A, B and E are courtesy of Chris Taylor. Other images were taken by RM.

638 Figure 2. Differences in the proportion (+/- 95% CI) of prey killed and consumed by spiders offered
639 different prey taxa in the field. Letters above bars indicate results of post hoc tests: species which
640 share a latter were not significantly different ($p > 0.05$).

641 Figure 3. Differences in behaviour of spiders towards different prey taxa offered to them in the field
642 in Experiment 1. Behavioural responses are characterised by means (+/- SEM) of two Principal
643 Components (PC1 and PC2) derived from Principal Components Analysis of the frequencies of seven
644 different behaviours observed in the field (see Table 3). High scores on PC1 indicate high frequencies
645 of most behaviours except “attack” and “left flower”. High scores on PC2 indicate high frequencies
646 of “attack” and low frequencies of “left flower”. Post hoc tests for PC1 showed that *P. dominula* and
647 *E. tenax* were significantly different from all other taxa ($p < 0.05$), but that there were no pairwise
648 differences amongst other taxa. Post hoc tests for PC2 showed that *P. dominula* was significantly
649 different from all other taxa, but that there were no pairwise differences amongst other taxa.

650 Figure 4. The effects of previous exposure to the aversive model *P. dominula*, and prey mimetic
651 accuracy, on the probability (+/- 95% CI) that spiders attacked prey of various taxa offered to them in
652 the laboratory in Experiment 2.

653 Figure 5. The effects of previous exposure to the aversive model *P. dominula*, and prey mimetic
654 accuracy, on spider behaviour when offered different prey taxa in the laboratory in Experiment 2.
655 Spider behaviour is described by mean PC1 scores (+/- SEM) derived from Principal Components

Analysis of the frequencies of eight different behaviours observed in the field (see Table 3), with high scores indicating spiders which were more active and less likely to attack prey.

Figure 6. The effects of previous exposure to the aversive model *P. dominula*, and prey taxon, on spider behaviour when offered different prey taxa in the laboratory in Experiment 2. Spider behaviour is described by mean PC2 scores (+/- SEM) derived from Principal Components Analysis of the frequencies of eight different behaviours observed in the field (see Table 3), with high scores indicating spiders which were more likely to attack prey, and less likely to flee from them.

Figure 7. The effects of prey type on the proportion of spiders (+/- 95% CI) that attacked real and artificial prey in the laboratory in Experiment 3. Prey types were: Natural Mimic (the accurate wasp-mimic *C. intermedium*; n = 13), Black Mimic (*C. intermedium* with its yellow stripes painted black; n = 11), Control Mimic (*C. intermedium* with its black stripes painted black; n = 9), Stripy Artificial (yellow clay with black stripes; n = 16), Yellow Artificial (yellow clay with yellow stripes; n = 16), Black Artificial (black clay with black stripes; n = 18) and Non-mimic (the non-mimetic fly *F. canicularis*; n = 15). Planned comparison tests are indicated above the bars for pairs of prey types of particular interest (NS: $p > 0.05$; * $p < 0.05$).

671 Table 1. Prey types used, their mimetic status, the experiments in which they were used, and their

672 ranked mimetic accuracy as perceived by humans (see main text).

Species / prey type	Mimetic status	Experiments	Mimetic Rank (1 = most accurate)
Polistes dominula (paper wasp) (L. 1758)	Model	All	NA
Chrysotoxum intermedium (Meigen 1822)	Wasp mimic	All	1
Syrphus ribesii (L. 1758)	Wasp mimic	1 & 2	2
Sphaerophoria scripta (L. 1758)	Wasp mimic	1 & 2	3
Episyrphus balteatus (De Geer 1776)	Wasp mimic	2	4
Apis mellifera (honeybee) (L. 1758)	Model	1	NA
Eristalis tenax (L. 1758)	Honeybee mimic	1	NA
Sarcophaga carnaria (L. 1758)	Not mimetic	1 & 2	5
Fannia canicularis (L. 1758)	Not mimetic	1, 2 & 3	6
C. intermedium paint control	Wasp mimic	3	NA
C. *intermedium* black	Not mimetic	3	NA
Artificial black	Not mimetic	3 & 4	NA
Artificial yellow	Not mimetic	3	NA
Artificial back and yellow stripes	Wasp mimic	3	NA

673

674 Table 2. Behaviours performed by *S. globosum* during experiments. Note that retreat and bungee

675 were not differentiated in Experiment 1.

Behaviour	Description
Leave Flower	Leaving the plant completely and not returning
Bungee	Jumping from the flower and dangling on a line of silk
Retreat	Moving away from the prey (and, in Experiment 1 only, bungeeing)
Display	Spreading and lifting fore-limbs while orientated towards prey
Approach	Orientation and movement towards the prey
Tickle	Gently touching the prey with its front legs
Grapple	Frantic touching of the prey and occasional jumping
Attack	Mounting and biting of the prey

676

677 Table 3. Results of Principal Components Analysis of behavioural responses by spiders to prey offered in the field in Experiment 1, and in the laboratory in
678 Experiments 2, 3 and 4. Correlation coefficients are shown indicating the strength and direction of the relationships between extracted Principal
679 Components (with Eigenvectors > 1.0) and the original variables describing the frequencies with which particular behaviours (see Table 2) were observed.
680 Note that retreat and bungee were not differentiated in Experiment 1.

	Experiment 1		Experiment 2			Experiment 3			Experiment 4		
Original variable	PC1	PC2	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Display	0.751	0.095	0.551	0.347	-0.044	0.772	0.268	0.230	0.821	0.304	0.097
Retreat	0.884	0.003	0.894	0.025	-0.114	0.849	-0.078	0.019	0.836	-0.270	0.053
Approach	0.874	0.145	0.620	0.190	0.550	0.749	-0.139	-0.01	0.836	-0.136	-0.070
Tickle	0.704	-0.189	0.717	0.140	-0.123	0.471	-0.160	-0.636	0.436	0.106	-0.174
Grapple	0.409	0.261	0.511	0.167	-0.498	0.634	0.402	0.297	0.698	0.395	0.219
Attack	-0.196	0.729	-0.459	0.713	0.059	-0.194	0.661	0.405	-0.109	0.809	-0.141
Bungee	NA	NA	0.236	-0.235	0.789	0.198	-0.751	0.285	0.332	-0.441	-0.452
Left flower	0.041	-0.745	0.165	-0.812	-0.193	-0.097	-0.621	0.541	-0.010	-0.213	0.839
Variance explained	40.17 %	17.44 %	31.96 %	17.85 %	15.54 %	32.34 %	20.87 %	13.55 %	35.88 %	15.57 %	12.79 %

681

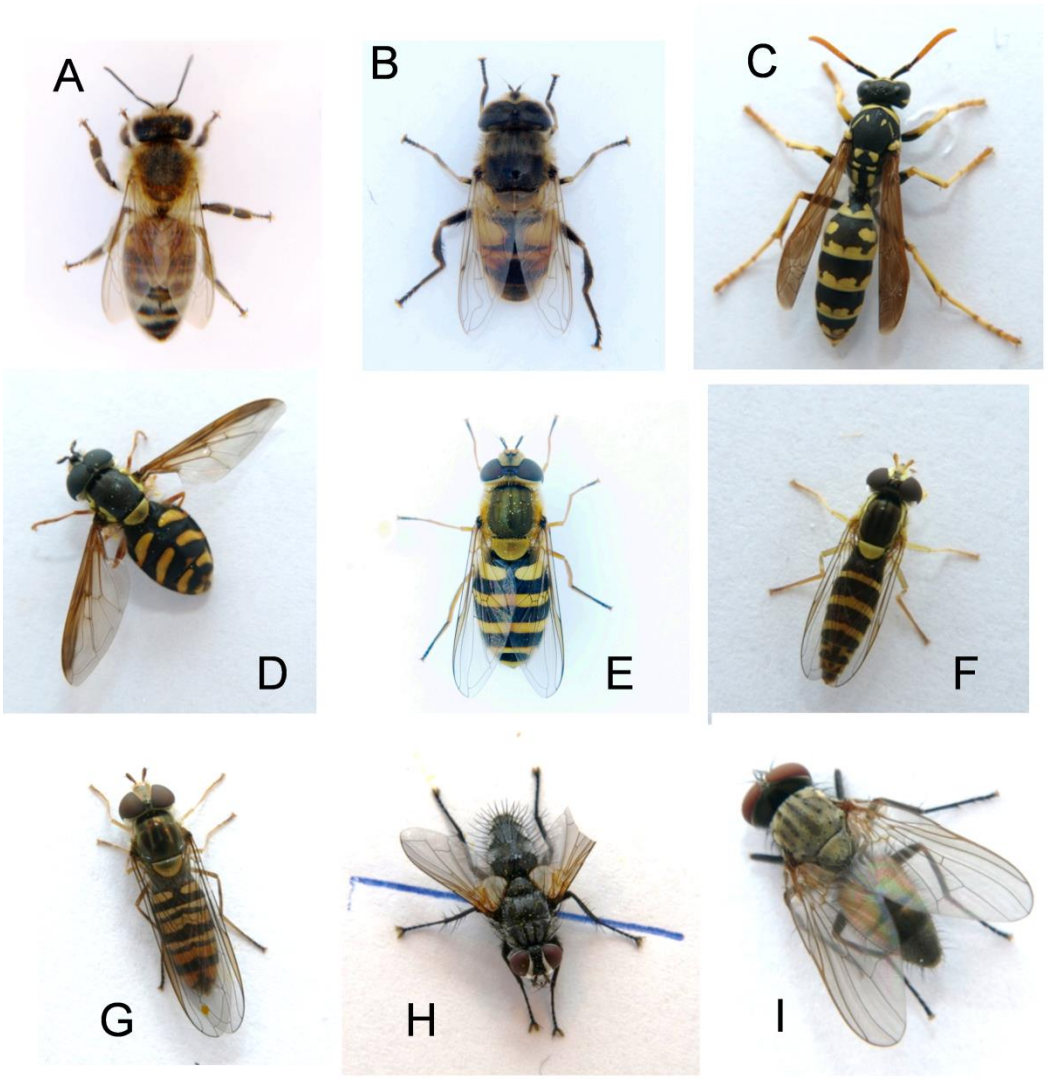
682 Table 4. Results of analysis of the effects of spider sex, prey taxon or mimetic accuracy, and treatment (whether spiders were exposed to a wasp before
683 encountering prey), and their interactions, on Principal Components (PCs) describing the behavioural responses of spiders to prey in the laboratory in
684 Experiment 2 (see Table 3). Models were fitted with a Gaussian error structure, and included either a fixed effect of prey taxon (GLMs), or a fixed effect of
685 mimetic accuracy and a random effect of prey taxon (GLMMs). Effects were assessed by F tests or Likelihood Ratio (LR) deletion tests during model
686 simplification. Significant p values are highlighted in bold.

	GLM with fixed effect of taxon									GLMM with fixed effect of accuracy								
	PC1			PC2			PC3			PC1			PC2			PC3		
Term	F	df	p	F	df	p	F	df	p	LR	df	p	LR	df	p	LR	df	p
Sex	0.061	1, 252	0.806	0.664	1, 251	0.416	3.782	1, 252	0.053	1.036	1	0.309	1.037	1	0.309	0.755	1	0.385
Taxon/Accuracy	0.924	5, 253	0.466	3.562	5, 253	**0.004**	5.573	5, 253	**< 0.001**	1.639	1	0.201	0.727	1	0.394	2.084	1	0.149
Treatment	0.062	1, 251	0.803	5.055	1, 252	**0.025**	0.498	1, 251	0.481	3.410	1	0.065	1.489	1	0.222	2.369	1	0.124
Sex x Taxon/Accuracy	0838	5, 240	0.524	0.466	5, 241	0.802	1.089	5, 246	0.367	0.756	1	0.385	2.301	1	0.129	0.327	1	0.568
Sex x Treatment	0.138	1, 245	0.710	0.001	1, 240	0.995	1.820	1, 241	0.110	0.162	1	0.687	0.027	1	0.869	2.394	1	0.122
Taxon/Accuracy x Treatment	1.213	5, 246	0.304	0.649	5, 246	0.663	0.798	5, 240	0.373	5.131	1	**0.024**	0.149	1	0.699	2.686	1	0.101
Sex x Taxon/Accuracy x Treatment	2.235	5, 235	0.052	0.910	5, 235	0.968	4.520	5, 235	**< 0.001**	2.348	1	0.125	0.206	1	0.650	0.858	1	0.354

687

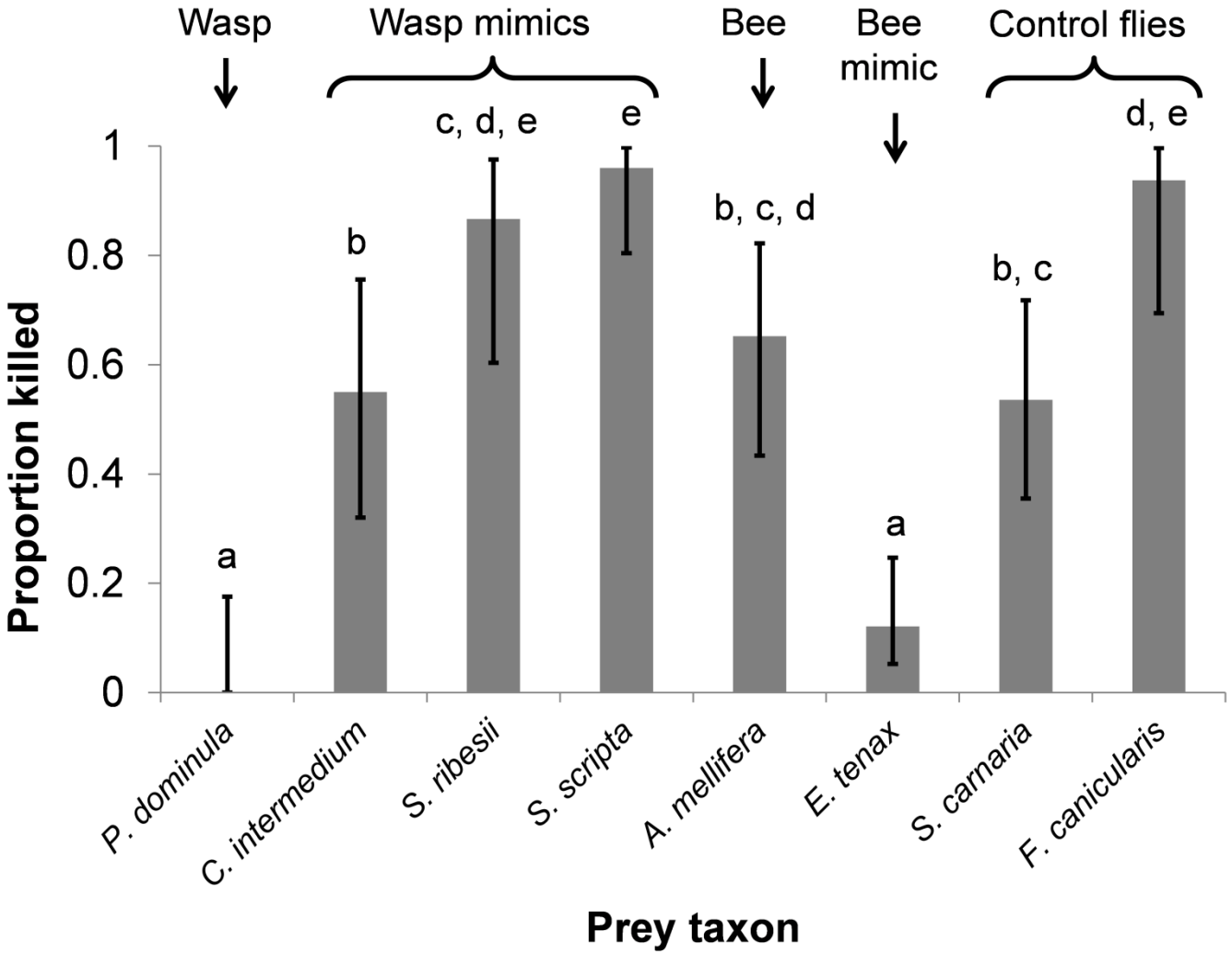
688 Table 5. Results of analysis of the effects of spider sex, prey odour and treatment (whether spiders
689 were exposed to a wasp before encountering prey), and their interactions, on Principal Components
690 (PCs) describing the behavioural responses of spiders to prey in the laboratory in Experiment 4 (see
691 Table 3). Effects were assessed by F-tests at deletion during simplification of a Gaussian general
692 linear model.

	PC1			PC2			PC3		
Term	F	df	p	F	df	p	F	df	p
Sex	13.745	1,236	< 0.001	0.245	1,236	0.622	1.638	1,236	0.202
Odour	1.320	3,232	0.269	0.031	3,232	0.993	0.855	3,233	0.465
Treatment	2.379	1,235	0.124	1.023	1,235	0.313	0.416	1,229	0.520
Sex x Odour	1.979	3,226	0.118	0.998	3,226	0.395	2.022	3,230	0.116
Sex x Treatment	0.014	1,225	0.905	0.105	1,225	0.746	3.223	1,228	0.074
Odour x Treatment	1.328	3,229	0.266	0.961	3,229	0.412	0.432	3,225	0.730
Sex x Odour x Treatment	2.025	3,222	0.111	0.871	3,222	0.457	4.130	3,222	0.243



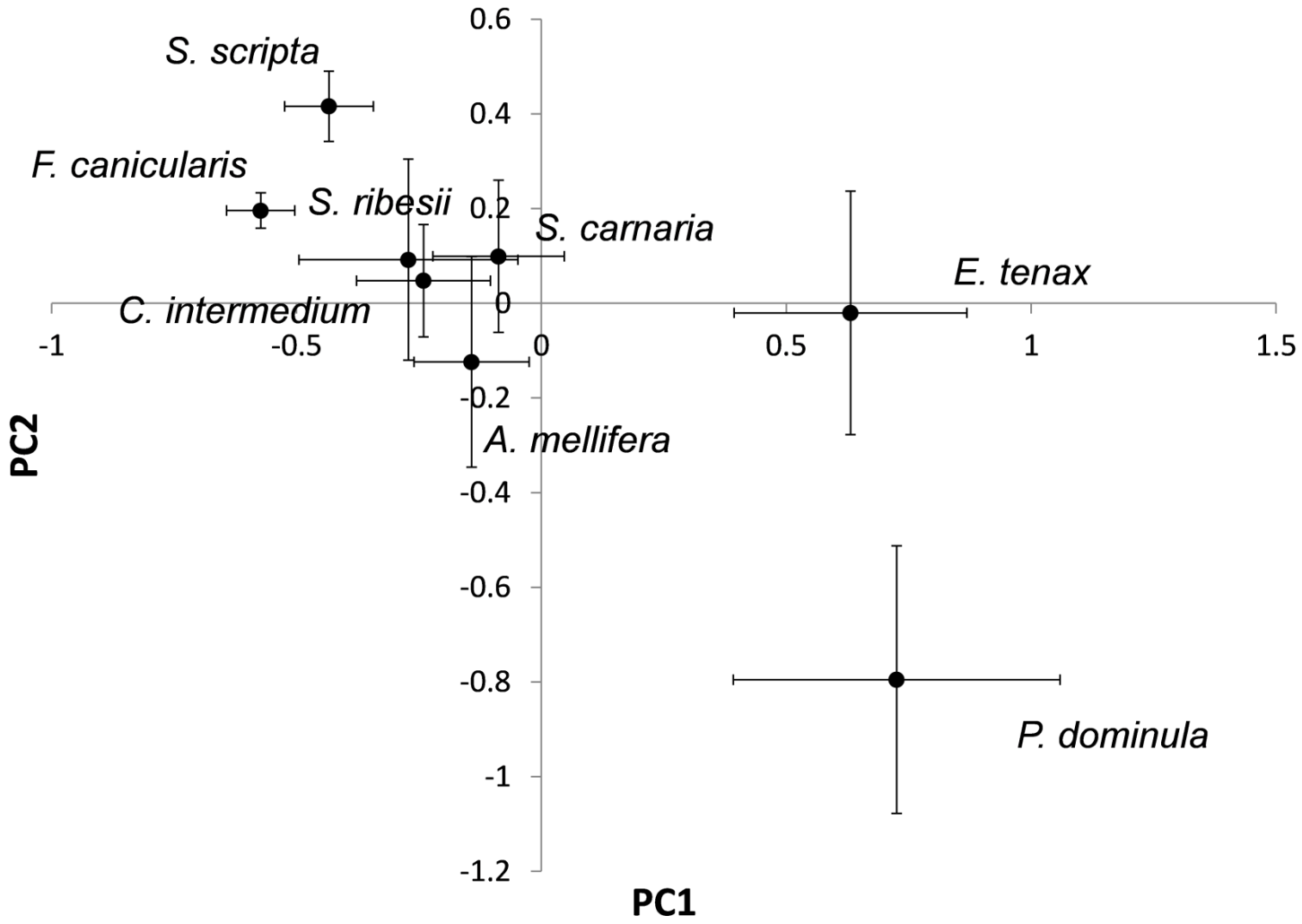
693

694 Figure 1



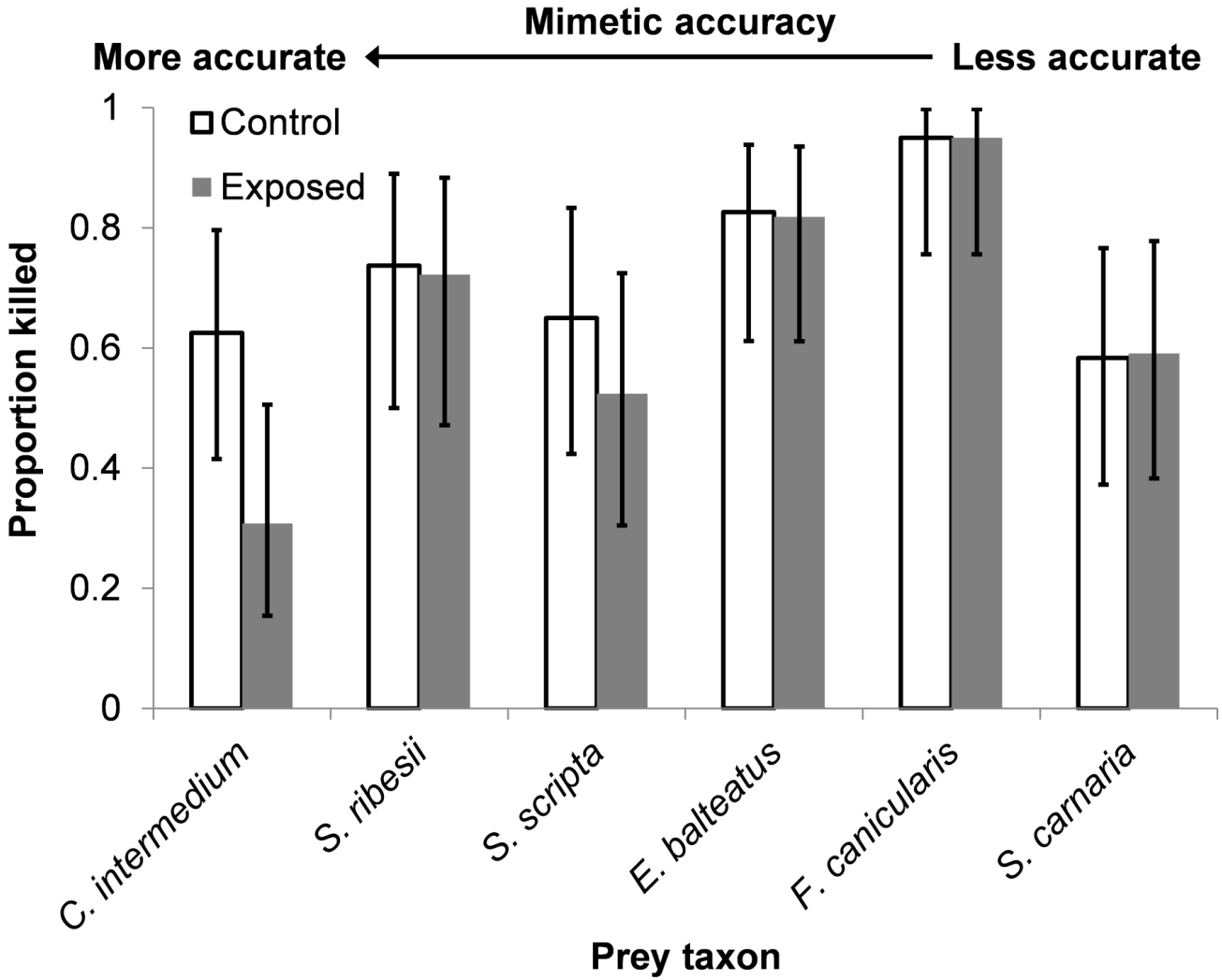
695

696 Figure 2



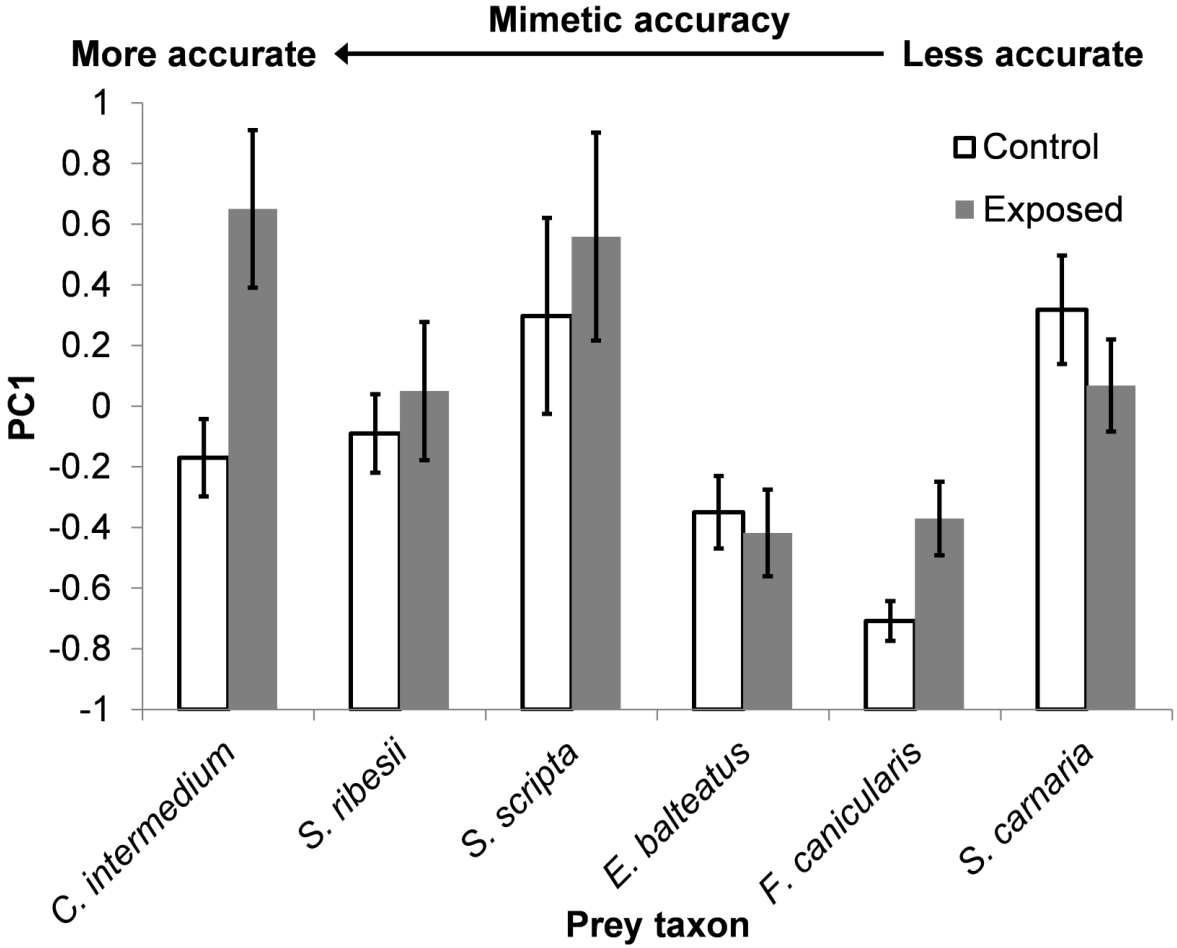
697

698 Figure 3



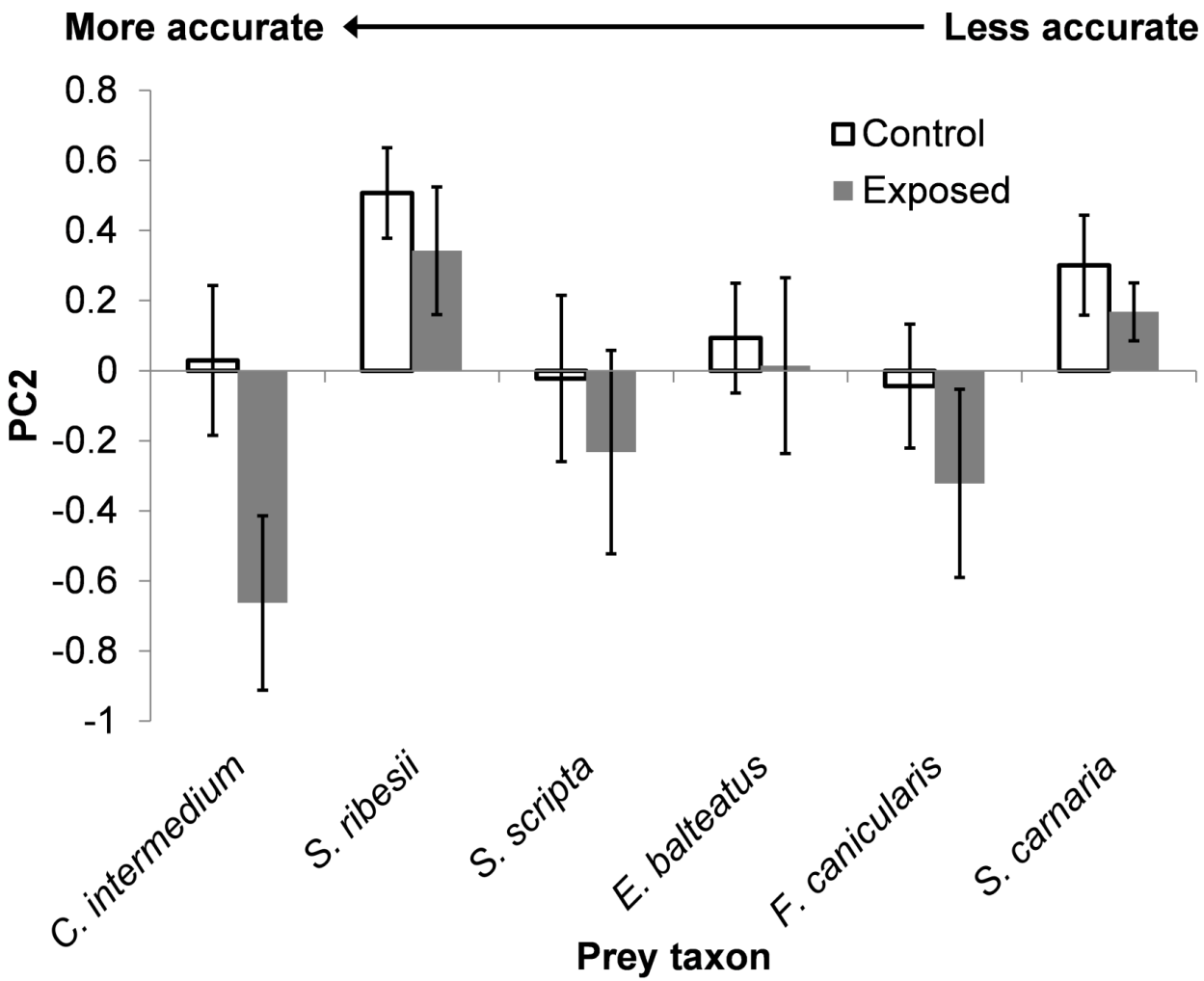
699

700 Figure 4



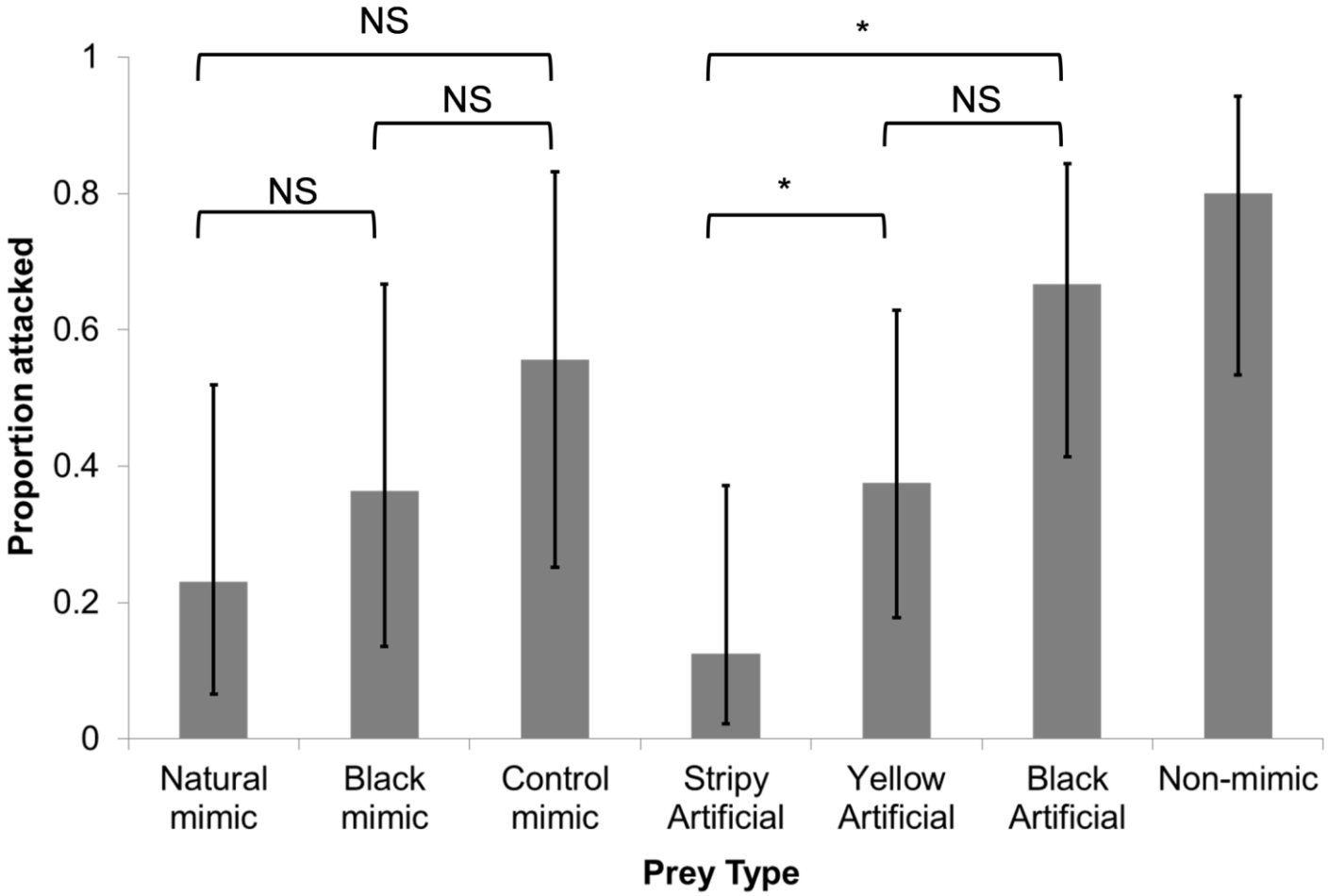
701

702 Figure 5



703

704 Figure 6



705

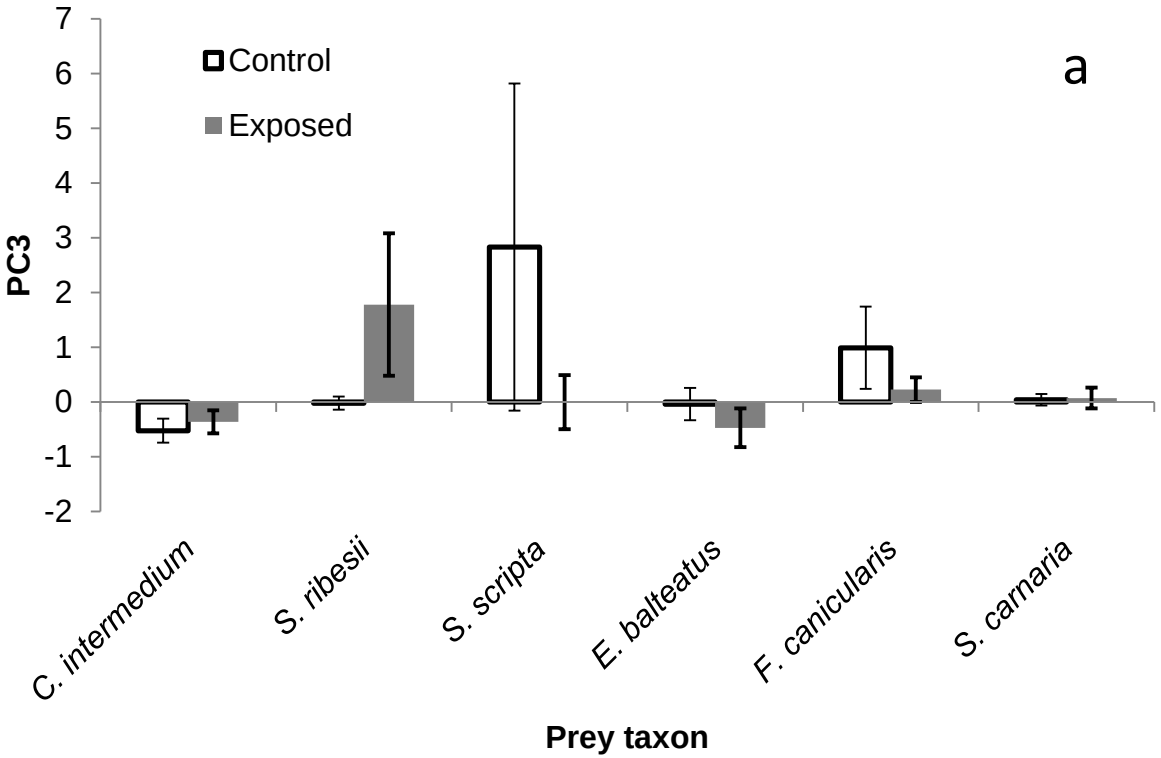
706 Figure 7

Supplementary information

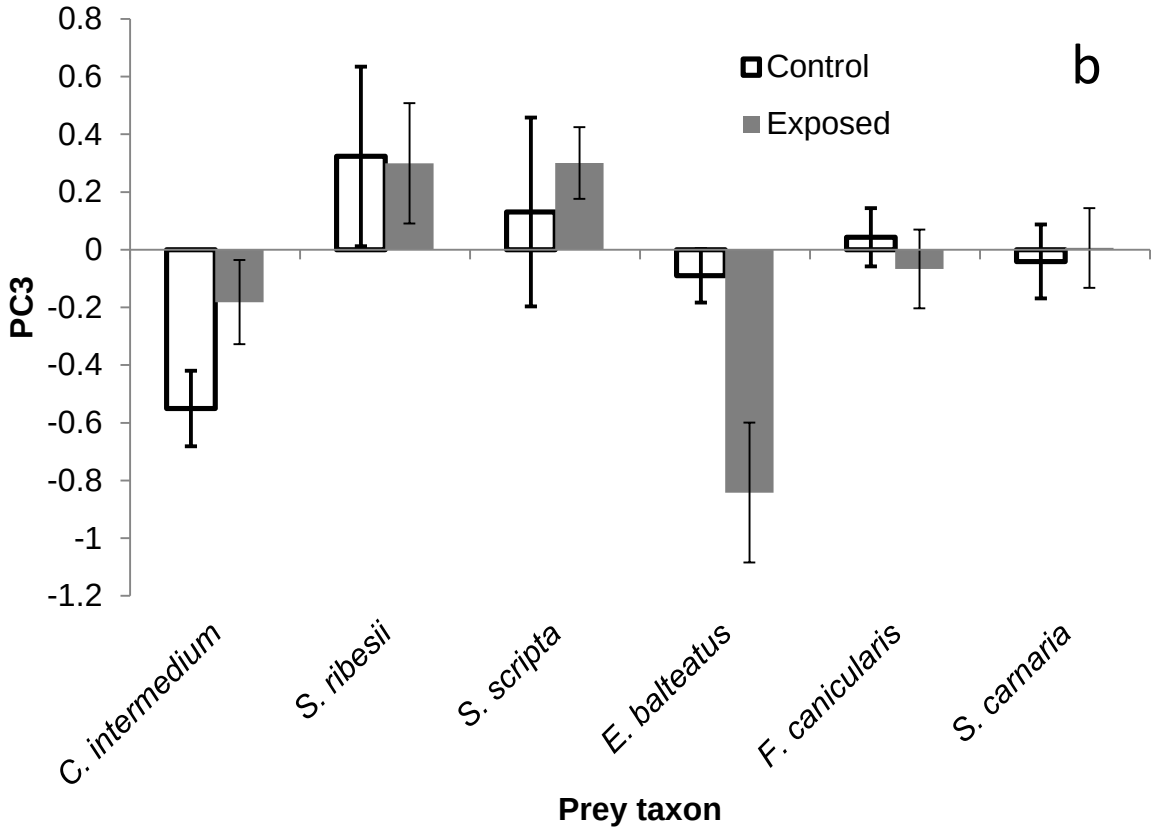
Figure legends

Figure S1. The effects of previous exposure to the aversive model *P. dominula*, and prey taxon, on the behaviour of male (a) and female (b) spiders, when offered different prey taxa in the laboratory in Experiment 2. Spider behaviour is described by mean PC3 scores (+/- SEM) derived from Principal Components Analysis of the frequencies of eight different behaviours observed in the field (see Table 3), with high scores indicating spiders which were more likely to "bungee" from the flower on which they were foraging.

Figure S2. The effects of prey type on the behaviour of spiders towards real and artificial prey in the laboratory in Experiment 3. Prey types were: Natural Mimic (the accurate wasp-mimic *C. intermedium*; n = 13), Black Mimic (*C. intermedium* with its yellow stripes painted black; n = 11), Control Mimic (*C. intermedium* with its black stripes painted black; n = 9), Stripy Artificial (yellow clay with black stripes; n = 16), Yellow Artificial (yellow clay with yellow stripes; n = 16), Black Artificial (black clay with black stripes; n = 18) and Non-mimic (the non-mimetic fly *F. canicularis*; n = 15). Spider behaviour is described by mean PC2 scores (+/- SEM) derived from Principal Components Analysis of the frequencies of eight different behaviours observed in the field (see Table 3), with high scores indicating spiders which were more likely to more likely to attack prey, and less likely to flee from them.


7
6
5
4
3
2
1
0
-1
-2
PC3
Control
Exposed
a
C. intermedium
S. ribesii
S. scripta
E. balteatus
F. canicularis
S. carnaria
Prey taxon

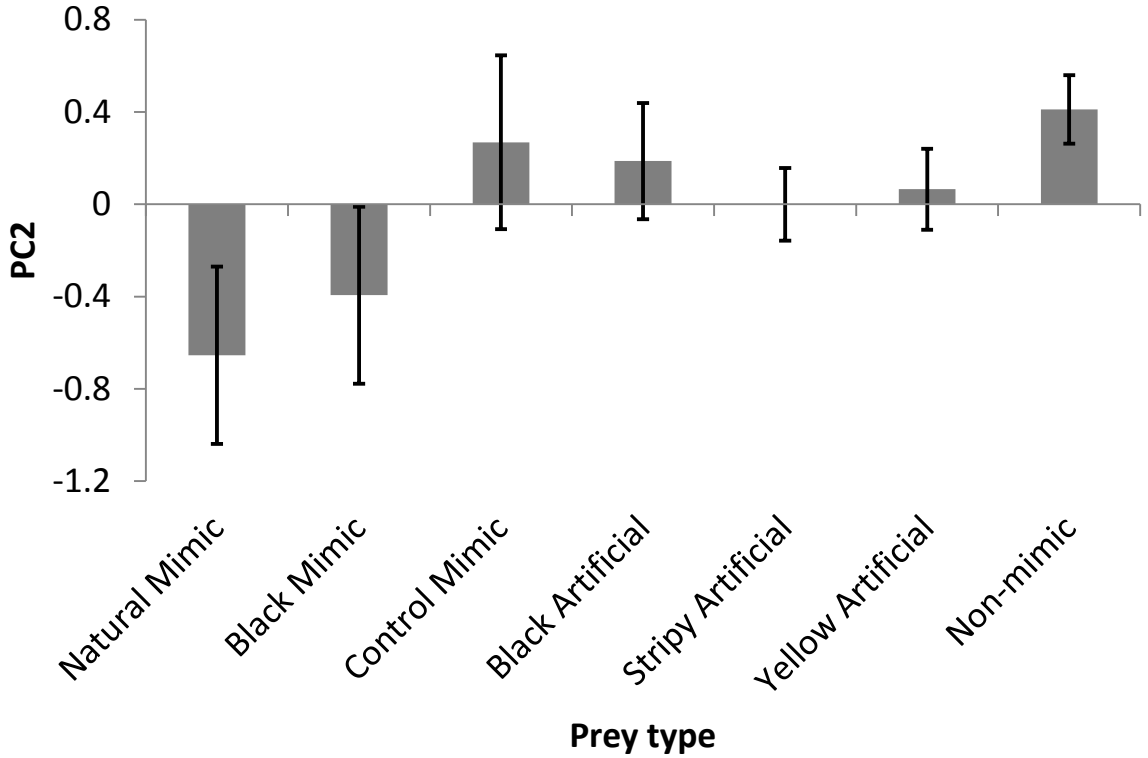
720



721

722 Figure S1.

723



724

725 Figure S2.

726